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Preliminary X-ray Diffraction Studies on 2 Fe-Ferredoxin from *Halobacterium* of the Dead Sea

The 2 Fe-ferredoxin from the *Halobacterium* of the Dead Sea has been crystallized. The space group is $P6_322$ with one protein molecule per asymmetric unit. The cell parameters are $a = b = 60{\cdot}6$ Å, $c = 127{\cdot}8$ Å. The crystals are stable under radiation and diffract to high resolution.

The unusual property of halophilic proteins to withstand high concentrations of salt, and in some cases the requirement of salt to maintain their conformation (Werber *et al.*, 1978*a*), has prompted us to study their three-dimensional structure.

Recently, a 2 Fe-ferredoxin has been isolated and purified from *Halobacterium* of the Dead Sea (Werber & Mevarech, 1978*a*), and other species of *Halobacterium* (Kerscher *et al.*, 1976), where it is present in large quantities—about 1% of the total protein. It was found to resemble chloroplast-type 2 Fe-ferredoxins in many properties such as optical characteristics and electron paramagnetic resonance spectrum. An immunochemical study (Geiger *et al.*, 1978) showed that halobacterial ferredoxin cross-reacted 15% with an algal ferredoxin (from *Spirulina maxima*), whereas it did not cross-react with 2 Fe-ferredoxins from a class to which *Escherichia coli* and other bacterial 2 Fe-ferredoxins belong. There is a high degree of similarity between the sequences of the halobacterial ferredoxins and that of the blue-green alga *Nostoc muscorum* (Hase *et al.*, 1978, and unpublished data). On the other hand, the halobacterial 2 Fe-ferredoxins possess some distinct characteristics: they are much more acidic than the chloroplast-type ferredoxins, as are most halophilic proteins (Werber *et al.*, 1978*a*), and have a higher redox potential: −345 mV as compared to −420 mV (Werber & Mevarech, 1978*a*; Kerscher *et al.*, 1976). The Mossbauer spectra for the reduced states have a narrower linewidth in the case of the halobacterial ferredoxin (Werber *et al.*, 1978*b*), as well as a lower temperature required to observe its electron paramagnetic resonance signal (Kerscher *et al.*, 1976; Werber *et al.*, 1978*b*) which imply shorter spin relaxation rates in halophilic ferredoxins, as well as possibly a weaker antiferromagnetic coupling. Finally, very distinct functional characteristics were found for these special ferredoxins: *Halobacterium* of the Dead Sea ferredoxin was found to be a cofactor in the reduction of nitrite to gaseous products, as part of a dissimilatory pathway of nitrate (Werber & Mevarech, 1978*b*), whereas *Halobacterium halobium* ferredoxin was found to be able to serve as a coenzyme of α-keto acid oxidoreductases (Kerscher & Oesterhelt, 1977).

In order to compare the structures of halobacterial to the chloroplast-type ferredoxins, for which there are preliminary reports of crystallizations (Ogawa *et al.*, 1977; Kunita *et al.*, 1978), we were interested in obtaining suitable crystals for the X-ray structure determination of ferredoxin from the *Halobacterium* of the Dead Sea. Our task was rendered easier by the availability of several hundred milligrams of very pure ferredoxin, which was obtained by the original purification procedure (Werber & Mevarech, 1978*a*).

We report here the crystallization of 2 Fe-ferredoxin from *Halobacterium* of the

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Dead Sea and preliminary crystallographic data. Initial attempts at crystallization produced red-colored crystals in the form of very fine needles by dialysis of ferredoxin at about 10 mg protein/ml, in pH 7 buffered 4 M-NaCl solutions, against 4 M-phosphate buffer at room temperature as well as at 4°C. These crystals were unsuitable for X-ray structure analysis due to their small size. In order to attempt to grow larger crystals we purified extensively about 200 mg of ferredoxin. One-ml samples of ferredoxin were then dialyzed at 12·5 and 25 mg/ml, in 4 M-NaCl buffer (pH 7·0), against 4 M-phosphate buffer at the same pH, yielding fine needles. The large needles—and other impurities—were then separated from the solution using Millipore filters (pore-size 0·45 μm) and from the filtrate beautiful red-colored hexagonal plate crystals grew, in about a week, up to 0·5 mm on each side.

Preliminary X-ray data were collected on precession photographs using Ni-filtered CuKα radiation from a sealed X-ray tube. The crystals belong to the hexagonal space group $P6_322$ with unit cell dimensions (as measured from the precession photographs) of $a = b = 60{\cdot}6$ Å, $c = 127{\cdot}8$ Å. The volume of the unit cell is 406,400 Å^3. Assuming one protein molecule in the asymmetric unit of molecular weight 14,000 (Werber & Mevarech, 1978*a*), one finds a specific volume of 2·42 Å^3/dalton which is within the average range found for other protein crystals (Mathews, 1968).

The crystals appear stable in the X-ray beam at room temperature for about 80 hours. Still photographs and small-angle precession photographs without a layer-line screen show that the diffraction pattern extends to at least 1·9 Å resolution, thus permitting a high-resolution structure determination.

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